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Original Article

Essential Oil, Phenol and Flavonoid Contents in Leaves and Fruits of *Prunus scoparia* (Spach) C.K. Schneid. Populations

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ABSTRACT

Medicinal plants are rich in secondary metabolites that constitute the composition of many drugs. The quantity and quality of these valuable materials are affected by environmental factors. The present study evaluated biochemical properties in leaves and fruits for three populations of *Prunus scoparia* (Spach) C.K. Schneid. Analysis of essential oil (EO) samples was performed by using GC and GC–MS. The results showed that total phenolic content (TPC) and total flavonoid content (TFC) of leaves in population 2 (Markazi) were 39.01 and 11.32 mg/g DW respectively. DPPH radical scavenging activity in population 2 was 45.5 % in leaves. Fruit EO content was 33% in population 1 (Lorestan). Heat map analysis distinguished two different clusters as one cluster for population 2 and another cluster for populations 1 and 3. The GC/MS analysis showed the main EO composition of fruits were Benzaldehyde (15.19-18.65%) followed by n-Hexadecanoic acid (14.67-18.3%), Benzyl Alcohol (4.12-6.42%), and 9-Octadecenoic acid (Z)-, ethyl ester (4.9-6.28%). In addition, the major EO profile for leaves were Neophytadiene (3.2-15%), dehydroaromadendrene (7.4-13.9%), Borneol (7.12-10.4%), cis-3-Hexenyl benzoate (6.4-9.5), trans-beta-Ionone (4.3-7.8%), Eugenol (2.3-7.3%), Benzyl benzoate (2.7-4.12%), and Di-epi-alpha-cedrene-(I) (2-5.5%).

INTRODUCTION

Prunus scoparia (Spach) C.K. Schneid. is a species of genus *Prunus*, which is commercially grown worldwide [1]. It is characterized by a subtropical Mediterranean climate, with moderate wet winters and dry summers [2,3]. Most cultivated almonds originate from the xeric environment of Central Asia [1]. In the regions, there has been identified over 30 wild species such as *P. scoparia* (Spach) C.K. Schneid, being the drought resistant species [2]. Approximately 20 species of wild almond have been identified in Iran, indicating that Iran is the main core of wild almond in the world. Iran due to various climates such as subtropical climate in the south of the country, temperate in the north part, Mediterranean in the west and center causes an extensive diversity of almond germplasm [3].

Plants growth and development in ecosystems are affected by genetic and environmental factors such as species (genetic), climate, edaphic, elevation and topography [4,5]. These parameters can significantly influence the quantity and quality of natural products in plants. Plant secondary metabolites have a remarkable role in the interaction of the plant with its environment and were described as defense system in plants [6]. These metabolites induce a dominant role in protection of plants from insect, pests, herbivores, phytopathogens and adaptation of the plants to the environment [6,7]. Among more than 25,000 secondary metabolites that have been identified in plants, phenolic compounds have been exploited as scavengers and inhibitors due to their antioxidant, antibacterial, anti-allergic, anti-inflammatory, anti-aging, and anti-tumor properties [6]. In addition, EOs is complex mixtures of volatile organic compounds generated as secondary metabolites. Environmental parameters affect the secondary metabolism of plants thereby altering essential oil (EO) composition and antioxidant activity [8,9]. They are mainly responsible for the distinctive odor of plants and also used as an eminent component in the cosmetic, food, and pharmaceutical industries. EOs composition is

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various between aromatic plant species and varieties and vary among the same variety from different geographic areas [10].

Different environmental conditions may influence the EO quality and quantity in medicinal plants. For example, the changes in EO quality and quantity have been reported in different ecotypes of ajowan [11], *Mentha longifolia* [12], *Artemisia umbelliformis* [13], and *Satureja bachtiarica* Bunge [14]. In addition, the phenolic compounds also may be changed under environmental conditions. For instance, there have been reported the changes in phenol and flavonoid in pomegranate [15], *Moringa oleifera* [*16*], *Olea europaea* [17].

As we know there is no valid document on the environmental variations on EO and phenolic contents in wild almond. The present study attempts to find this gap in the wild almond populations. Therefore, the main objective of present study was (i) to evaluate the EO content and composition in leaves and fruits of three different populations (Tehran, Markazi, and Lorestan) of *P. scoparia* (Spach) C.K. Schneid., and (ii) to assess the differences of phenol and flavonoid contents of leaves and fruits in the wild almond populations. The hypothesizes are that secondary metabolites are different among three populations *P. scoparia* (Spach) C.K. Schneid., which comprise distinguished ecotypes. The findings of present study could be useful in determining the desirable population according to it secondary metabolites such as EO and phenolic contents.

MATERIAL AND METHODS

Plant material and site description

The leaves and fruits of *P. scoparia* (Spach) C.K. Schneid. were taken on a linear transect in three different sites in June 2019. The experimental sites were Tehran (51º 05′ 51″ E and 35º 05′ 51″ N), Makazi (50º 32′ 21″ E and 33º 47′ 36″ N), and Lorestan (48º 15′ 22″ E and 33º 30′ 31″ N). Soil properties of three experimental sites have been described in table 1.

Treatment details

This work was done based on completely randomized design (CRD) with 10 replications in three populations of *P. scoparia* (Tehran, Markazi, and Lorestan) during 2019.

Determination of total phenolic content (TPC)

Folin–Ciocalteu reagent was applied to determine the TPC. 100 µl of the MeOH solution of the measured weight of investigated plant 1–10 (2.54, 2.58, 2.25, 4.03, 4.80, 2.13, 4.62, 1.47, 1.58, 15.05 mg/mL, respectively) were mixed with 0.75 mL of Folin–Ciocalteu reagent and let to remain at 21 ºC for 5 min. 0.75 ml of $NaHCO_3$ solution was appended to the mixture. After 95 min at 21 ºC, absorbance was measured at 725 nm. Standard curve was calibrated by Gallic acid (0–100 mg/L). The calibration curve manifested the linear regression at r> 0.99, and the outcomes were demonstrated as mg GAE/g DW [18].

Isolation procedure

EO content of wild almond was quantified based on the method described by European Pharmacopoeia for oil production [19]. The100 gr of dried aerial parts were subjected to hydro-distillation for 3 h, employing a Clevenger-type apparatus.

Gas chromatography

The analysis of GC was conducted according to Shimadzu GC-9A gas chromatography equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm) to calculate oil components [20].

Table 1 Soil properties in three different populations of *P. scoparia* (Spach) C.K. Schneid.

Site	Clay (%)	Silt (%)	Sand (%)	T.N.V (%)	Total N (mg/kg)	K (mg/kg)	P (mg/kg)	pH	EC (dS/m)
Tehran	32.0	18.5	49.5	2.5	0.098	217	7.0	7.59	0.497
Markazi	32.5	13.0	54.5	15.5	0.014	217	14.7	7.60	3.030
Lorestan	22.5	10.0	67.5	2.5	0.133	165	15.7	7.30	0.511

Gas chromatography-mass spectroscopy

The analyses of GC-MS were performed in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm) to identify all oil components. Oven temperature was 50-240 ºC at a 4 ºC/min rate, transfer

line temperature of 260 ºC, helium as a carrier gas with a velocity of 31.5 cm/s, split ratio 1:60, ionization energy of 70 eV, 1 s scan time, and 40-300 amu. of mass range [20].

Determination of total flavonoid content (TFC)

Aluminum chloride method was applied to measure the TFC method [21]. Briefly, the mixture containing 0.5 mL of sample and 300 µL of $NaNO_2$ (1:20 w/v) was vortexed for 10 s and left to stand at 24 º C for 5 min. After that, the reaction mixture was changed by 300 µL of $AlCl_3$ (1:10 w/v), 2 mL of NaOH (1 M) and 1.9 mL of distilled water, and then vortexed for 10 s. The absorbance was determined at 510 nm. Quercetin concentrations ranging from 0 to 1200 µg/mL were prepared and linear fit was used for calibration of the standard curve.

Radical scavenging activity

Free radical scavenging activities of extracts was measured using a DPPH radical described by Brand-Williams *et al*. [22]. The 0.1 ml of the extract solution was mixed with 1.0 ml of DPPH solution and 4 ml of methanol. The mixture was kept at ambient temperature for 30min prior to measurement of the absorbance at 517 nm. The scavenging rate was determined as follow:

Eq. (1): DPPH scavenging% = [1/ (A 517 nm, sample – A 517 nm, control)] × 100.

Statistical analysis

The data (n = 10) were subjected to one-way analysis of variance (ANOVA) and using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). When statistical significance ($p < 0.05$) was detected, the mean values subjected to Duncan's multiple range tests.

RESULTS

Phenol in leaves and fruits

TPC in leaves and fruits was significantly changed. TPC of leaves ranged from 33.92 to 39.01 $mgg^{-1}DW$ (Fig. 1a). Fruit TPC changed from 11.54 to 14.47 $mgg^{-1}DW$ (Fig 1b). leaves and fruits TPC were 39.01 and 14.47 $mgg^{-1}DW$ respectively in population 2 (Markazi).

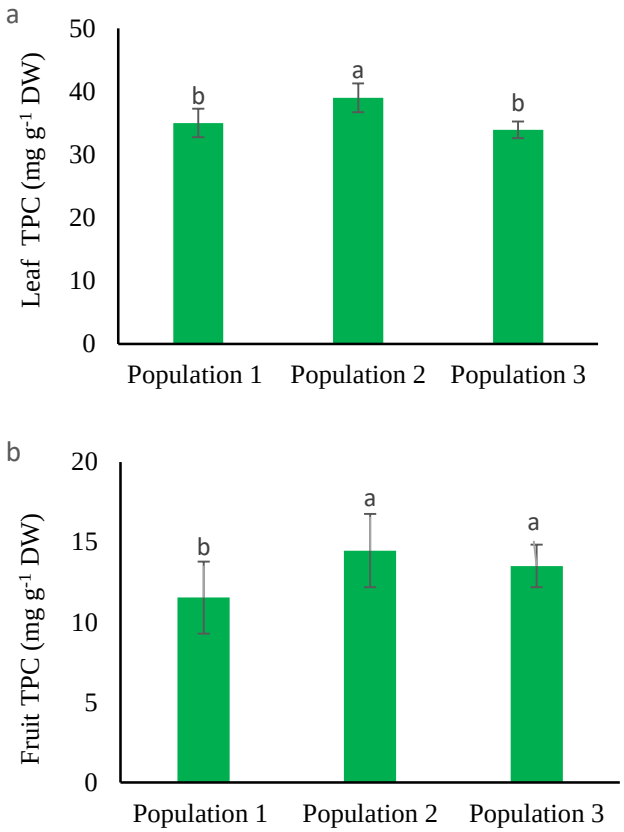

Fig. 1 Total phenolic content (TPC) of three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

TFC in leaves and fruits

TFC was changed in different populations. Leaves TFC ranged from 8.34 to 11.32 mg g^{-1} DW (Fig. 2a). TFC of fruits changed from 11.54 to 14.47 mg g^{-1} DW (Fig. 2b). Leaves and fruits TFC were 11.32 and 14.47 mg g^{-1} DW in population 2 respectively.

DPPH scavenging activity in leaves and fruits

DPPH scavenging activity in both leaf and fruit was different. DPPH scavenging of leaves changed from 34.7 to 45.5% (Fig. 3a). Fruits' DPPH scavenging ranged from 35.4 to 46.2% (Fig. 3b). Leaves and fruits DPPH scavenging were 45.5 and 46.2% in population 2.

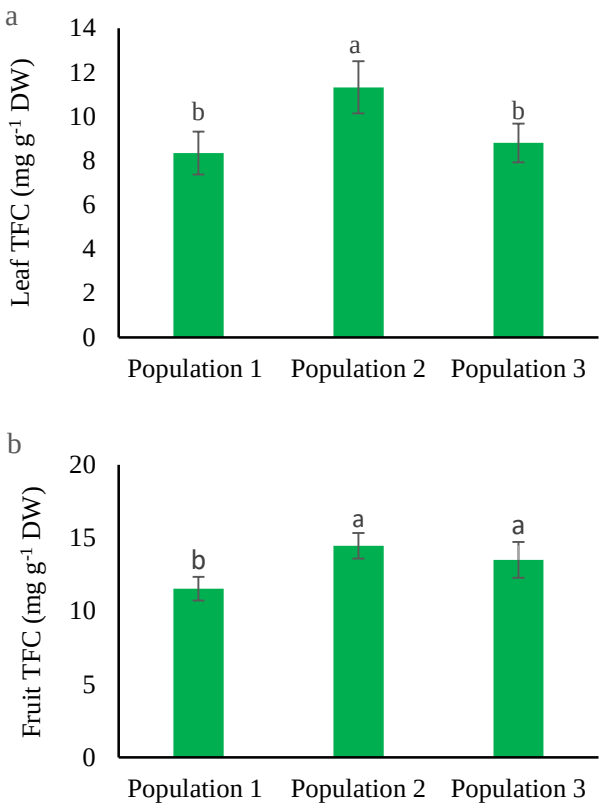

Fig. 2 Total flavonoid content (TFC) of three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

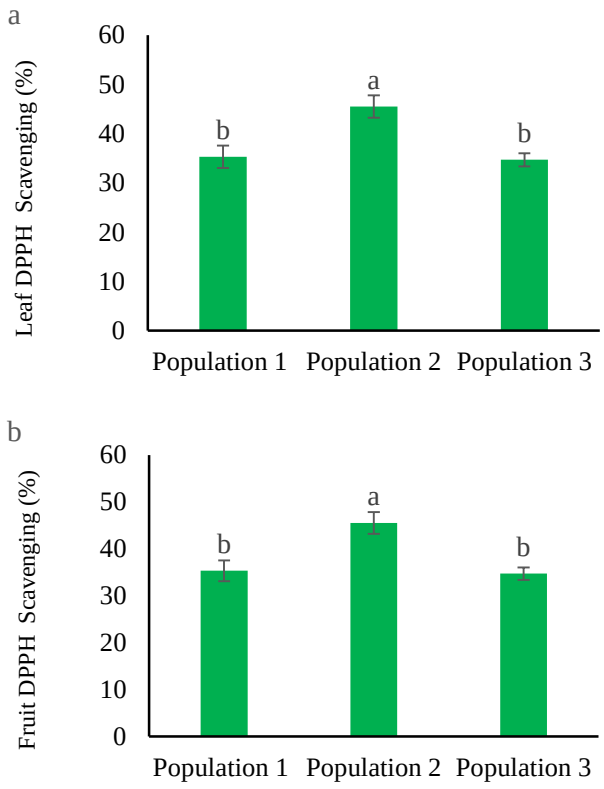

Fig. 3 DPPH scavenging activity in three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

EO content in leaves and fruits

EO response was different in both leaf and fruit. Leaves EO changed from 26 to 33 % (Fig. 4a). Fruits EO ranged from 36 to 48 % (Fig. 4b). Leaves EO were 33 and 26% in population 1 and population 3 respectively.

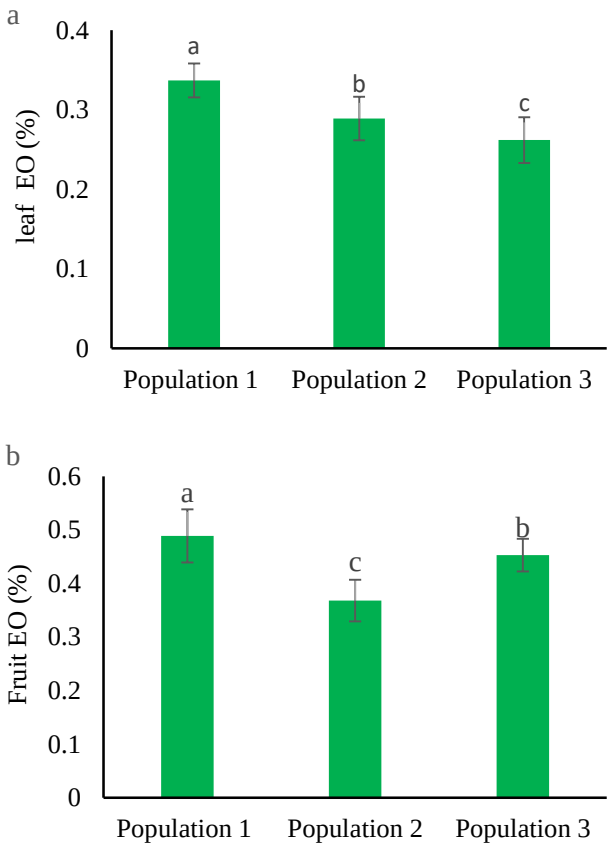

Fig 4. Essential oil (EO) content in three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

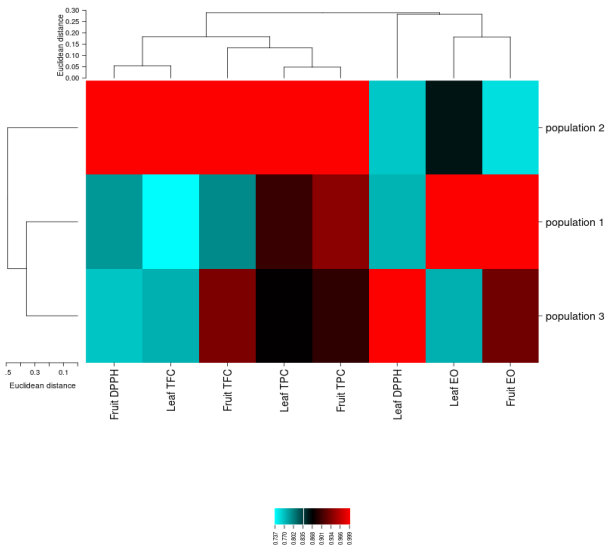

Fig. 5 Heat map analysis for the studied traits of three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

EO composition

The GC/MS analysis showed different responses between the populations for EO profile (Tables 2 and 3) The main EO composition of fruits were Benzaldehyde (15.19-18.65%) and n-Hexadecanoic acid (14.67-18.3%) followed by Benzyl Alcohol (4.12-6.42%), Benzoic acid (2.91-4.31%), n-Hexadecane (1.98-3.15), n-Heptadecane (1.98-3.7%), n-Octadecane (3.18-5.71%), Isopropyl Myristate (3.11-4.32%), 9-Octadecenoic acid, methyl ester (3.8-5.3%), and 9-Octadecenoic acid (Z)-, ethyl ester (4.9-6.28%). The main EO composition for leaves were Neophytadiene (3.2-15%), dehydroaromadendrene (7.4-13.9%), Borneol (7.12-10.4%), cis-3-Hexenyl benzoate (6.4-9.5), trans-beta-Ionone (4.3-7.8%), Eugenol (2.3-7.3%), Benzyl benzoate (2.7-4.12%), Di-epi-alpha-cedrene-(I) (2-5.5%), Benzaldehyde (1.5-3.3%).

Table 2 Essential oil composition of tree fruits in three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

Compounds name	R.I.	Population 1	Population 2	Population 3
Furfural	830	-	-	0.301
Benzaldehyde	952	15.19	22.6	18.65
Benzyl Alcohol	1026	6.42	4.12	5.87
Menthone	1148	0.38	0.12	-
Nonanoic acid	1267	-	0.67	0.54
Thymol	1289	0.39	0.47	0.43
n-Tridecane	1300	0.56	-	0.23
Benzoic acid	1310	3.18	2.9	4.31
Dodecane, 4,6-dimethyl-	1325	0.62	0.98	0.54
Dodecane, 4-methyl-	1326	1.76	-	1.2
n-Tetradecane	1388	1.63	0.98	-
Tetradecane, 5-methyl-	1400	0.4	0.52	0.54
Decane, 1-iodo-	1433	0.65	0.55	-
Tetradecane, 4-methyl-	1455	1.36	1.26	1.65
n-Pentadecane	1500	1.55	0.98	1.13
Phenol, 2,4-bis(1,1-dimethylethyl)-	1581	1.81	1.23	0.98
Pentanoic acid, 2,2,4-trimethyl-3- carboxyisopropyl, isobutyl ester	1581	0.895	0.895	0.895
n- Hexadecane	1600	3.155	1.98	2.45
Ar-tumerone	1668	1.08	1.21	0.87
Benzoic acid, 2-ethylhexyl ester	1674	0.853	0.98	0.89
n- Heptadecane	1700	3.069	1.98	2.12
Pentadecane, 2,6,10,14-tetramethyl-	1712	1.1	0.98	-
Benzyl benzoate	1759	1.563	1.45	1.21
Heptadecane, 2-methyl-	1764	0.34	0.12	-
Heptadecane, 3-methyl-	1774	0.67	0.34	0.55
n- Octadecane	1800	4.33	5.71	3.18
Isopropyl Myristate	1836	3.11	3.98	4.32
Hexahydro farnesyl acetone	1833	0.88	0.82	0.89
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9- diene-2,8-dione	1916	0.94	0.55	0.75
Hexadecanoic acid, methyl ester	1921	1.44	0.98	1.21
Diisobutyl phthalate	1922	2.33	2.98	2.16
n-Hexadecanoic acid	1959	16.8	14.67	18.3
Hexadecanoic acid, ethyl ester	1991	4.53	3.77	3.27
9-Octadecenoic acid, methyl ester	2058	3.86	4.78	5.3
9,12-Octadecadienoic acid	2092	1.56	-	0.87
9-Octadecenoic acid (Z)-, ethyl ester	2174	5.54	6.28	4.9
Total	-	93.96	91.83	90.98

Table 3 Essential oil composition of tree leaves in three populations (Lorestan: population 1, Markazi: population 2; Tehran: population 3) of *P. scoparia* (Spach) C.K. Schneid.

Compounds name	RI	Population 1	Population 2	Population 3
Benzaldehyde	952	3.3	1.5	2.47
Benzyl Alcohol	1026	2.06	0.9	1.6
PARA CYMENYL	1099.11	0.45	0.23	0.43
Linalool	1107.5	1.64	1.35	2.23
Hotrienol	1112.30	0.7	0.9	0.3
Alpha.-Campholenal	1138.77	0.2	0.9	-
Nonanol	1180.51	0.2	0.8	0.7
Borneol	1186.81	10.4	7.12	9.23
p-Cymen-8-ol	1202.26	0.37	0.49	0.36
ALPHA. TERPINEOL	1208.28	2.75	2.5	2.9
Safranal	1214.07	0.37	0.12	0.23
p-Menth-1-en-9-al	1232.05	1.2	0.5	0.9
linalyl acetate	1256.44	0.34	0.65	0
Trans-Geraniol	1260.0	0.64	0.55	0.78
Perillol	1306.53	0.27	-	-
4-vinyl-2-methoxy-phenol	1329.12	0.43	-	-
Di-epi-.alpha.-cedrene-(I)	1337.74	2	5.5	3.2
Eugenol	1368.70	7.35	5.3	2.3
Damascenone	1390.05	0.82	0.84	0.23
Dehydroaromadendrene	1397.46	13.93	11.3	7.4
Caryophyllene	1428.44	1.3	0.6	0.07
4-(2,4,4-Trimethyl-cyclohexa-1,5- dienyl)-but-3-en-2-one	1441.37	2.29	2.9	2.7
5,9-Undecadien-2-ol, 6,10-dimethyl-	1457.60	0.8	1.5	0.8
Humulene	1465.68	0.53	0.12	0.23
Trans-.beta.-Ionone	1490.47	4.33	6.3	7.8
Farnesene	1506.04	0.64	0.87	0.61
3,3,5,6-Tetramethyl-1-indanone	1522.31	0.49	0.17	0.63
Nerolidol	1566.32	2.68	2.23	4.68
Cis-3-Hexenyl benzoate	1586.77	8.77	9.5	6.4
Hexyl benzoate	1592.75	3.67	4.12	5.78
Farnesene epoxide, E-	1603.67	1.55	0.98	1.01
Ar-tumerone	1668	1.53	1.93	1.7
Bisabolol oxide	1668.96	1.7	1.5	1.4
Tetradecanol	1682.47	0.95	0.93	0.87
Benzyl benzoate	1759	3.15	4.12	2.7
Neophytadiene	1828.33	3.28	3.7	15
Hexahydrofarnesyl acetone	1833	1.11	1.5	0
Phenethylbenzoate	1883.24	0.98	0.87	0.93
Farnesyl acetone	1914.20	0.25	0.29	-
Diisobutyl phthalate	1922	-	1.7	2.70
Isophytol	1941.12	1.21	4.8	2.3
Dibutyl phthalate	1969.14	-	0.54	0.57
PALMITIC ACID ETHYL ESTER	1989.68	1.62	3.2	1.9
Total	-	92.27	95.82	96.04

Heat map analysis showed the two distinguished clusters; population 2 as a cluster and populations 1 and 3 as another cluster. As seen by Fig. 5, the high weights (red) of traits were found in population 2. EO content and yield in population 1 was significantly higher compared to other treatments (Fig. 5).

DISCUSSION

Phenolics and flavonoids have been widely proposed as developmental regulators and/or signalling

molecules in plants exposed to a wide range of environmental stimuli [23,24]. Our data revealed that TPC and TFC were recorded in plants from the population 2 (Markazi site). This finding also suggests the photoprotective role of polyphenols and flavonoids [25]. Accumulation of polyphenols has been reported in *Thymus vulgaris* under direct sunlight in comparison with shady conditions [26]. Additionally, flavonoid accumulation was reported in *Silene littorea* populations under higher solar radiation [27]. On the other hand, the equal amounts of TPC and TFC in the populations 1 and 3 imply that accumulation of these compounds may be related to duration of light rather than its intensity. For further scrutiny, the results revealed that the amount of TFC was higher in the population 2, which is attributed to the higher contents of kaempferol in this site. Similarly, increased TPC and TFC were reported in *Centaurea glomerata*. Flavonoids derivatives such as other phenolic compounds are the main factors that determine the plant's antioxidant activity. Farhat *et al*. [28] reported that the vegetative and reproductive stages of plants can be characterized by the TPC. The content of TPC of extracts has shown variability during the different sites. Souhir *et al*. [29] showed the change TPC and TFC under different climatic conditions.

The DPPH assay is the most common method to characterize the activity of free radical savaging of phytochemical antioxidants [5]. A similar antioxidant activity assay by DPPH in *Juglans regia* L. was also reported by Ghasemi et al. [30]. The high content of phenolic compounds enhances the rate of hydrogen transfer to free radicals and the inhibitory strength [31]. About 28 % increase was obtained in leaves of population 2 Heat map analysis confirmed the correlation between TPC, TFC, and DPPH scavenging activity. The high antioxidant activity of *P. scoparia* (Spach) C.K. Schneid. extract might be attributed to the high TPC and TFC at all populations that confirmed the strong antioxidant activity of these compounds as previously suggested by Afshari and Rahimmalek [32], Saki et al. [33], and Farhadi et al. [5]. The high content of phenolic compounds enhances the rate of hydrogen transfer to free radicals and the inhibitory strength [33].

The changes in EO content were observed in three different populations of *P. scoparia*. The populations are different in geological substrates and soil types. The ecological diversity of localities leads the species to the adaption for specific conditions that can developed different ecotypes and chemotypes. The production of EO is correlated to metabolic conditions and reflects the interactions between plants and environmental factors followed by adaption and higher survival rate [31]. Fejér *et al*. [34] showed that the most important factors affecting the accumulation of EO were pH and humus content among soil properties and altitude among topographic factors. Differences in photosynthesis capacity [33], environmental factors [31] as well as size and density of glandular trichomes [35] change the biosynthesis and accumulation of volatile oil by time. Padilla-González et al. [35] noted that the expression and activity of involved genes and enzymes in plant secondary metabolism are varied at different cultivars. The high EO content in population 1 is due to its environmental and soil factors. Accordingly, Mollaei *et al*. [36] showed the change in EO content and quality under environmental factors particularly temperature and altitude. Geng *et al*. [37] reported that benzaldehyde (62.52%), benzoic acid (14.80%), and n-Hexadecanoic acid (3.97%) were the most abundant components of bitter almond. We also obtained Benzaldehyde (15.19-18.65%) as one of the main component of EO leaves and fruits and benzoic acid and n-Hexadecanoic acid as main components of EO fruits. The present work revealed the change in main EO composition under environmental factors. Bajalan *et al*. [38] showed the variation between different accessions of *Rosmarinus officinalis* L. The change in EO profile is related to genotype and ecological factors [33].

CONCLUSION

Environmental factors can significantly alter the secondary metabolites of medicinal plants. The results of our study showed the variation of essential oil quality and quantity, and also phenol and flavonoid content. In respect to the antioxidant capacity and according to heat map analysis, at all populations, the extracts revealed the highest phenolic content and antioxidant activity. EO percentage in the fruits was significantly higher compared to that in leaves. To obtain the higher EO content, we should select the site similar to Lorestan (population 1).

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